

# Determination of Stability Constants and Gibbs Free Energies of Cefotaxime-Zn(II) Complex at Different Temperatures

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## ABSTRACT

Cefotaxime is a synthetic  $\beta$ -lactam antibiotic that is active against Gram-negative and Gram-positive bacteria. Cefotaxime is able to chelate metal ions due to the presence of C=O, NH<sub>2</sub>, COOH, COOR, NH and NO electron donating groups. The stability constants and Gibbs free energies of cefotaxime-Zn(II) were determined colorimetrically at 25 and 40 °C using continuous variation and mole ratio methods. The formation of Zn(II) complex with cefotaxime was studied colorimetrically at an absorption maximum of 430 nm at different temperatures. The data showed that Zn(II) and cefotaxime combine in the molar ratio of 1:2 at pH 7.4 with ionic strength maintained using 0.1M KNO<sub>3</sub>. Calculated stability constants values were  $1.96 \times 10^5$  and  $1.28 \times 10^5$  using continuous variation method and  $1.11 \times 10^5$  and  $1.11 \times 10^5$  using mole ratio methods at 25 and 40 °C respectively. Calculated  $\Delta G^\circ$  for the complex were  $-3.01 \times 10^4$  and  $-3.06 \times 10^4$  J using continuous variation method and  $-2.88 \times 10^4$  J and  $-3.02 \times 10^4$  J using mole ratio method at 25 and 40 °C respectively. The stability constant results suggested that cefotaxime used in the study is a good chelating agent and can be an efficient antidote in the therapy of Zn(II) overload or poisoning.

**KEYWORDS:** Cefotaxime, zinc, complex, stability constant, Gibbs free energy

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## 1. INTRODUCTION

The human body has an elaborate mechanism for management and regulation of key trace metals circulating in bloodstream and in the cells. Essential trace metals from our diet are transported into the blood when there is depletion in blood levels. Essential trace metals are also transported into cells if there is inadequacy in the cellular levels. Excreted of trace metals occur if blood and cellular levels are sufficiently overloaded. When there is systemic failure in function, abnormality in levels and ratios of trace metals can develop. One of such trace-metal imbalances is depressed zinc. The concentration of zinc is important for healthy living [1]. The amount of Zn distributed in the human body is about 2-4 grams [2]. Zinc is found mainly in the brain, muscle, bones, kidney and liver, with the highest concentrations in the prostate and parts of the eye [3]. Zn is the second most dominant transition metal in biological systems after iron and it is the only metal that appears in all enzymatic classes [2,4]. Many cellular metabolic processes requires the involvement of Zn [5]. It was reported that 10% of human proteins potentially complex with zinc, including hundreds which transport and traffic zinc [6,7]. Catalytic activity of more than 200 enzymes requires Zn mineral [6,7].

It is essential in boosting immunity [7,8], facilitate wound healing [8] and the synthesis of protein.

Cefotaxime (Figure 1) is a synthetic  $\beta$ -lactam antibiotic that is active against Gram-negative and Gram-positive bacteria. These  $\beta$ -lactam antibiotics inhibit the synthesis of the peptidoglycan layer from the cell wall [9]. The  $\beta$ -lactam ring in the structure of cefotaxime helps the antibiotic to bind to the enzymes that synthesize the peptidoglycan layer and inhibits the process. The mechanism of bacteria resistant to  $\beta$ -lactams antibiotics is due to the production of  $\beta$ -lactamase enzymes that damages the  $\beta$ -lactam ring so that the antibiotic cannot bind to peptidoglycan layer [9]. The antibiotic cefotaxime belongs to the third generation cephalosporins that possess higher potency against bacteria compared to the first and second generation cephalosporins.

For several decades, chelating agents have been used as antidote to combat metal poisoning [10]. Biological friendly complexing agents have been used effectively to chelate metals in patients with metal overload [10]. The synthesis and antibacterial activity of cefotaxime complexes of Mn(II),

Fe(II), Co(II), Ni(II), Cu(II) and Cd(II) (Figure 2) have also been reported [11]. Many authors have reported the study of stability constant of drug-metal complexes [12 – 14]. However, to the best of authors knowledge, stability constants and Gibbs free energies of cefotaxime – Zn(II) complex at different temperatures have yet not appeared in the literature. These stability constants are useful to study the effects of cefotaxime on trace elements and mineral metabolism. It is possible that changes in trace element and mineral concentration induced by cefotaxime can be an efficient antidote in the therapy of zinc overload or poisoning. In this context, the aim of this study was to assess stability constants and free energy change of cefotaxime-Zn(II) complex at 25 and 40 °C respectively.

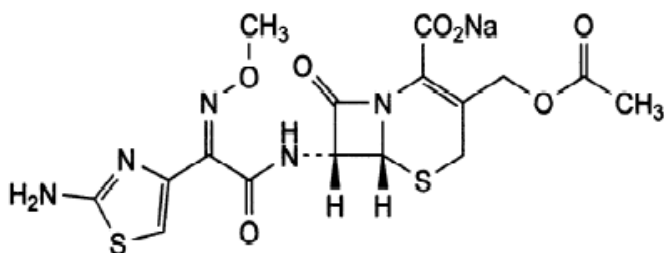


Figure 1: Chemical structure of cefotaxime

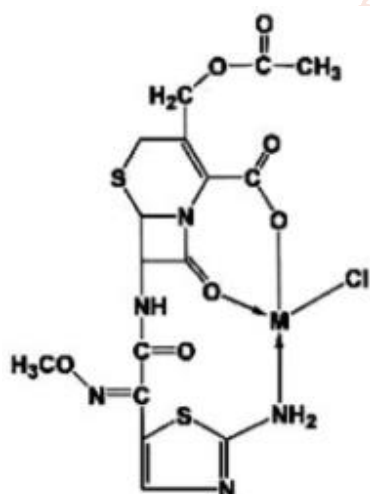


Figure 2: Tentative structure of cefotaxime metal complexes, M = Mn(II), Co(II), Ni(II), Cu(II) and Cd(II)

## 2. Materials and methods

### 2.1. Instrumentation

Absorbances were measured using auto colorimeter ME-51. Orion Versa Star Pro pH Benchtop meter (VSRAR10 series) was used for pH measurements.

### 2.2. Reagents

All the chemicals used were of analytical grade purity. Cefotaxime was purchased from Nitin Life sciences Limited, Indian. ZnCl<sub>2</sub> was purchased from Merck Germany. Double-distilled water was used throughout the experiment.

### 2.3. Preparation of 2 x 10<sup>-2</sup> M ZnCl<sub>2</sub>

ZnCl<sub>2</sub> (2.73 g, 20 Mmol, M. Wt. = 136.29 g/mol) was dissolved in freshly distilled in a 250 cm<sup>3</sup> beaker and was made up to the mark in a 1000 cm<sup>3</sup> volumetric flask.

### 2.4. Preparation of 2 x 10<sup>-2</sup> M cefotaxime

Cefotaxime (9.1094 g, 20 Mmol, M. Wt. = 455.47 g/mol) was dissolved in freshly distilled in a 250 cm<sup>3</sup> beaker and was made up to the mark in a 1000 cm<sup>3</sup> volumetric flask.

### 2.5. Procedure for continuous variation method

ZnCl<sub>2</sub> (2 x 10<sup>-2</sup> M) (0, 1, 2, 3, 4, 5, 6 cm<sup>3</sup>) was pipetted out and transferred into seven 50 cm<sup>3</sup> volumetric flasks. Cefotaxime (2 x 10<sup>-2</sup> M) (6, 5, 4, 3, 2, 1, 0 cm<sup>3</sup>) was added, respectively to the Zn(II) solution so that the mole fraction remained constant. The pH adjusted to 7.4 and ionic strength maintained constant by using 0.1 M KNO<sub>3</sub>. Their absorbance were measured at 430 nm (maximum absorbance of the complex) and at a temperature of 25 and 40 °C, respectively.

### 2.6. Procedure for mole ratio method

ZnCl<sub>2</sub> (2 x 10<sup>-2</sup> M) (2 cm<sup>3</sup>) was pipetted out and transferred into each of the seven 50 cm<sup>3</sup> volumetric flasks. Cefotaxime (2 x 10<sup>-2</sup> M) (1, 2, 3, 4, 5, 6, 7 cm<sup>3</sup>) was added to each of the Cu(II) solution respectively. Wavelength of maximum absorbance of the complex (430 nm) was noted against blank reagent CuSO<sub>4</sub>. Their absorbance was measured at 430 nm and at a temperature of 25 and 40 °C, respectively.

### 2.7. Calculation of stoichiometry mole fraction, stability constant and free energy

The stoichiometry mole fraction (SMF) of the complex using continuous variation method was calculated using equation 1 [15].

$$SMF = \frac{m}{1 - m} \quad 1$$

Where m is the mole fraction of metal ion.

Equation 2 [15] was applied to the calculation of stability constant.

$$K_{cef-Zn} = \frac{1 - \alpha}{m^m \cdot n^n (\alpha)^{m+n} (C)^{m+n-1}} \quad 2$$

Where C is the concentration of the complex at stoichiometry point,  $\alpha$  is the degree of dissociation, m and n are the corresponding stoichiometric coefficients of metal and ligand respectively.

The degree of dissociation ( $\alpha$ ) was calculated using equations 3, 4 and 5 [15].

$$A_{\alpha} = A_o - A_{max} \quad 3$$

$$A_{max} = \epsilon b C \quad 4$$

$$\alpha = \frac{A_{\alpha}}{\epsilon b C} \quad 5$$

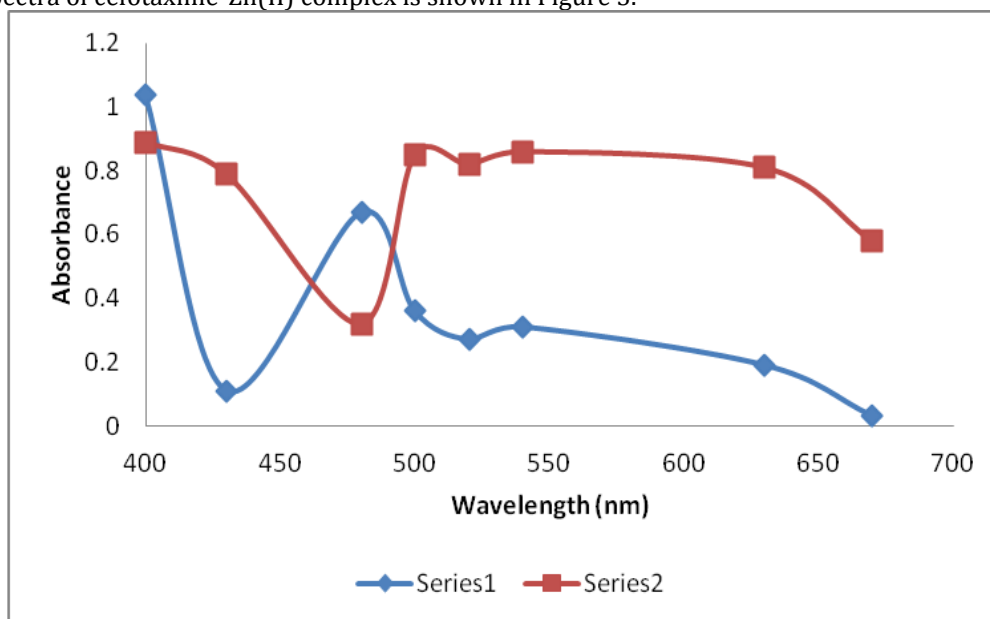
Where  $A_{max}$  is absorbance value of the maximum at experimental curve that represents the maximum quantity of the complex that is formed.  $A_o$  is absorbance value corresponding to the intersect point of the theoretical straight lines.  $A_{\alpha}$  is the absorbance value of the part of dissociated concentration of complex.  $\epsilon$  is molar absorptivity, b is cell thickness, C is a concentration of complex at stoichiometry point.

The Gibbs free energy was calculated using equation 6.

$$\Delta G^{\theta} = -RT \ln K \quad 6$$

### 3. Results and discussion

The electronic spectra of cefotaxime-Zn(II) complex is shown in Figure 3.



**Figure 3: Absorption spectra of  $\text{ZnCl}_2$  ( $2 \times 10^{-2} \text{ M}$ ) (series 1) and cefotaxime-Zn(II) complex ( $2 \times 10^{-2} \text{ M}$ ) (series 2)**

The electronic spectra were measured from 400 – 670 nm. The electronic spectrum of cefotaxime-Zn(II) complex showed  $\lambda$  maximum at 430. At the wavelength,  $\text{ZnCl}_2$  have a weak absorbance. Therefore, this wavelength was used for the measurement of absorbance in the determination of the stability constants and free energies.  $\text{ZnCl}_2$  absorbs maximally at wavelength of 400 nm. It was observed that cefotaxime-Zn(II) complex gave a white, water soluble complex. In aqueous solution, zinc-aquo complex is a labile complex because water behaved as a weak ligand. Cefotaxime displaced water from zinc-aquo to form a stable cefotaxime - Zn(II) complex. Similar labile aquo complexes were also reported by Tirmizi and co-workers in their study of famotidine-Cu complex and cimetidine-Ni complex [13, 14]. Tella and co-workers reported labile aquo complex in their study of Dapsone-Cu(II) stability constants [10].

**Table 1: Experimental data of cefotaxime-Zn(II) complex at 430 nm by continuous variation method**

S/N	$\text{ZnCl}_2$ ( $2 \times 10^{-2} \text{ M}$ )	Cefotaxime ( $2 \times 10^{-2} \text{ M}$ )	Mole fraction of Zn(II)	Absorbance at 430 nm	
				25 °C	40 °C
1	0.00	6.00	0.00	0.00	0.00
2	1.00	5.00	0.17	0.05	0.04
3	2.00	4.00	0.33	0.22	0.21
4	3.00	3.00	0.50	0.41	0.42
5	4.00	2.00	<b>0.66</b>	<b>0.50</b>	<b>0.50</b>
6	5.00	1.00	0.83	0.18	0.19
7	6.00	0.00	1.00	0.06	0.09

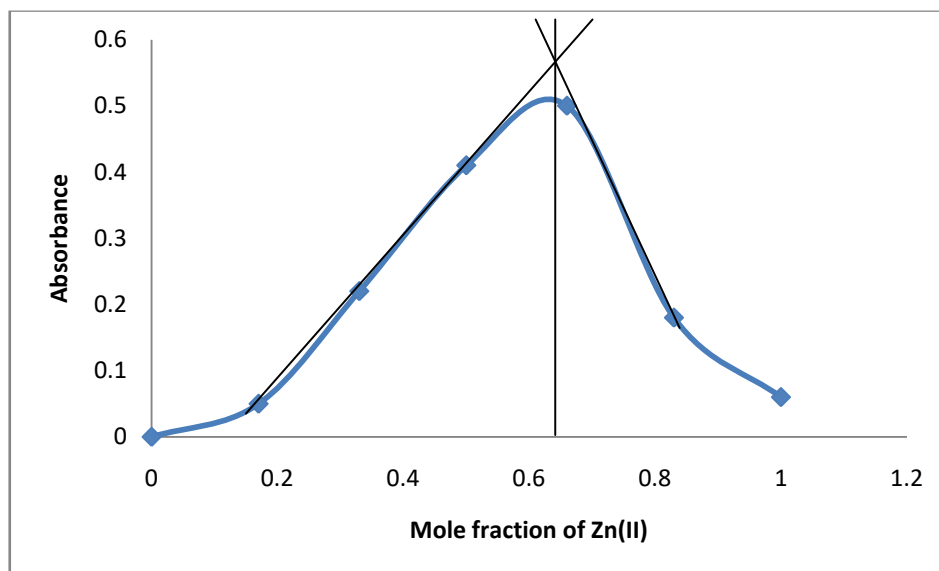


Figure 4: Job's curves for stability constants of equimolar solutions at 25 °C

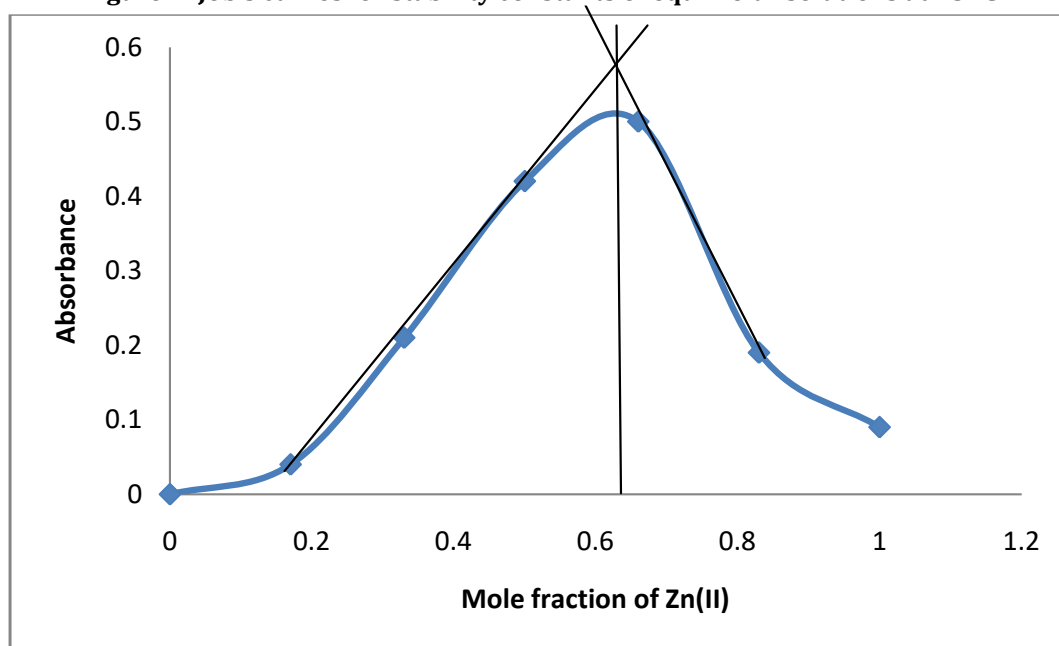
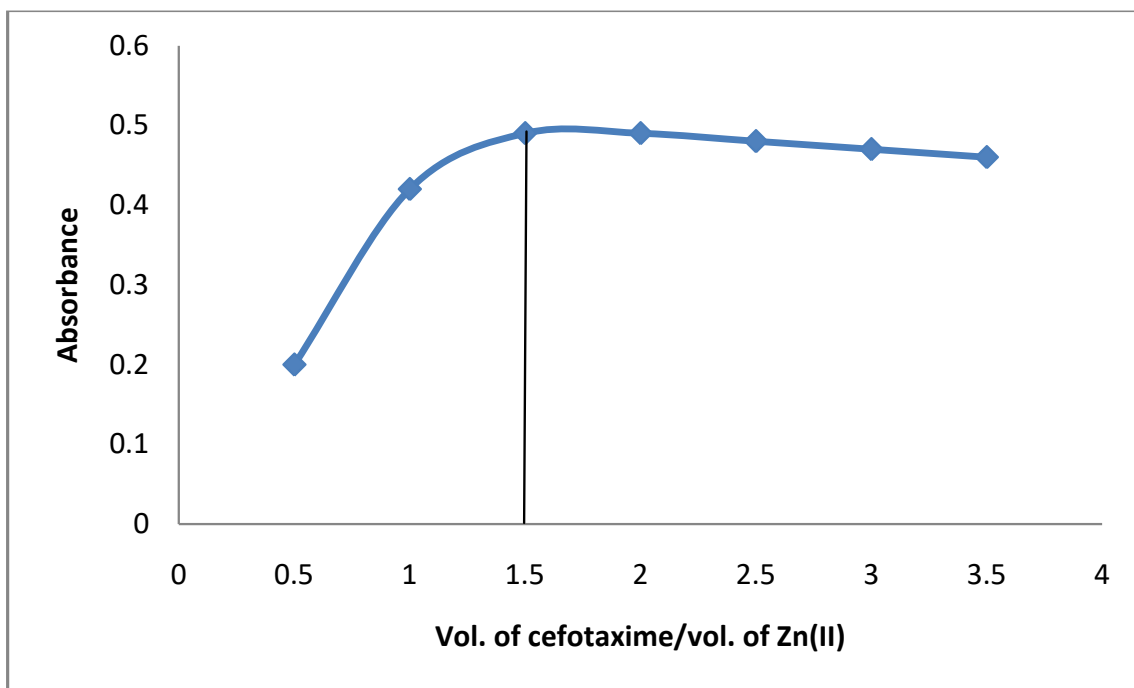


Figure 5: Job's curves for stability constants of equimolar solutions at 40 °C

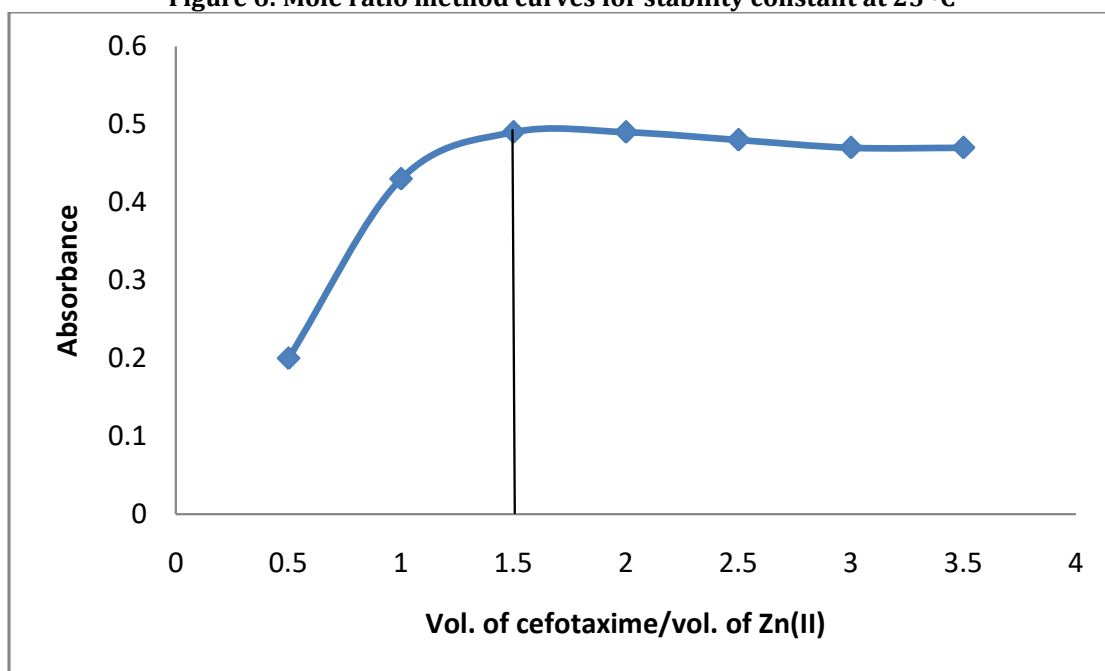
The extrapolated value at the point of cross-section on continuous variation plot (Figures 4 and 5) corresponded to the total absorbance of the complex, indicating that the complex formation process has been completed. The mole fraction of Zn(II) at the point of intersection are 0.64 and 0.63 at 25 and 40 °C respectively. Application of equation 1, yields SMF of 1.77 ( $\approx 2$ ) and 1.70 ( $\approx 2$ ) at 25 and 40 °C respectively. This corresponded to metal:ligand ratio of 1:2. This is in agreement with the report of Reiss and co-workers [9] where 1:2 metal:ligand ratio was proposed for Zn (II) complexes with cefotaxime-derived Schiff base.

Table 2: Experimental data of cefotaxime-Zn(II) complex at 430 nm by mole ratio method

S/N	ZnCl <sub>2</sub> (2 x 10 <sup>-2</sup> M)	Cefotaxime (2 x 10 <sup>-2</sup> M)	Vol of Cefotaxime/ vol of Zn(II)	Absorbance at 430 nm	
				25 °C	40 °C
1	2.00	1.00	0.5	0.20	0.20
2	2.00	2.00	1.0	0.42	0.42
3	2.00	3.00	<b>1.5</b>	<b>0.49</b>	<b>0.49</b>
4	2.00	4.00	2.0	0.49	0.49
5	2.00	5.00	2.5	0.48	0.48
6	2.00	6.00	3.0	0.47	0.47
7	2.00	7.00	3.5	0.46	0.47



**Figure 6: Mole ratio method curves for stability constant at 25 °C**



**Figure 7: Mole ratio method curves for stability constant at 40 °C**

The metal:ligand ratio of the complex was evaluated from the point where this curve changes its slope. The extrapolated value at the point of cross-section on mole ratio plot (Figures 6 and 7) corresponded to the total absorbance of the complex, indicating that the complex formation process has been completed. The vol. of cefotaxime/vol. of Zn(II) at the point of intersection are  $1.5 \approx 2.0$  and  $1.5 \approx 2.00$  at 25 and 40 °C respectively. This corresponded to metal:ligand ratio of 1:2. This was in agreement with the proposed structure reported in literature [9].

**Table 3: Calculated stability constant values and  $\Delta G^\circ$  for cefotaxime-Zn complex**

S. No	Method	Metal: ligand ratio	Stability constant (log K)		$\Delta G^\circ$ (J)	
			25 °C	40 °C	25 °C	40 °C
1	Continuous variation	1:2	$1.96 \times 10^5$	$1.28 \times 10^5$	$-3.01 \times 10^4$	$-3.06 \times 10^4$
2	Mole ratio	1:2	$1.11 \times 10^5$	$1.11 \times 10^5$	$-2.88 \times 10^4$	$-3.02 \times 10^4$

The stability constant values showed that the complex was stable both at room temperature and higher temperature. The values of the stability constants obtained from continuous variation compared well with that of mole ratio method. It can be seen from the Table 3 that the values obtained by both methods are in fair agreement. Increasing the temperature of complexation from 25 to 40 °C had no effect on the stability constant. The values of the stability constants were positive, this suggested that the complex was stable. Similar positive values of stability constant of complexes were reported by Tirmizi and co-workers [13, 14] using continuous variation and mole ratio methods. Waranyoupalin and co-workers also reported positive



stability constant values using continuous variation and mole ratio methods [16]. The results of stability constant suggested that cefotaxime could be effective in chelation therapy against Zn(II) toxicity. The negative values of the free energies suggested that the complexes were formed spontaneously. Both Job's and mole ratio methods supported the metal to ligand ratio of 1:2 as evidenced from the results obtained by Reiss and co-workers [9].

## Conclusion

Cefotaxime is a third generation cephalosporin. It formed a reasonably stable complex with Zn(II). Job's method of analysis corresponded well with the values obtained using mole ratio method of analysis. The Job's continuous variation and mole ratio methods data showed that Zn(II) and cefotaxime combine in the molar ratio of 1:2. The stability constant results suggested that cefotaxime used in the study is a good chelating agent and can be an efficient antidote in the therapy of Zn(II) overload or poisoning.

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